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Origin of the Putrescine-Producing Ability of the Coagulase-Negative Bacterium *Staphylococcus epidermidis* 2015B[▽]

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A multiplex PCR method, aimed at the detection of genes associated with biogenic amine production, identified the *odc* gene encoding ornithine decarboxylase in 1 of 15 strains of *Staphylococcus epidermidis*. The ability of the positive strain, *S. epidermidis* 2015B, to produce putrescine *in vitro* was demonstrated by high-performance liquid chromatography (HPLC). In this strain, the *odc* gene was detected on plasmid DNA, suggesting that the ability to form putrescine is carried by a mobile element, which explains the fact that the trait is strain dependent within the *S. epidermidis* species. A 6,292-bp nucleotide sequence harboring the putative *odc* gene was determined. *S. epidermidis* ornithine decarboxylase (ODC) showed 60 to 65% sequence identity with known ODCs of Gram-positive as well as Gram-negative bacteria. Downstream of the *odc* gene, a gene encoding a putative amino acid transporter was found that shared 59% sequence identity with the ornithine/putrescine exchanger (PotE) of *Escherichia coli*. Cloning and expression of the *potE* gene of *S. epidermis* 2015B in *Lactococcus lactis* demonstrated that the gene product transported ornithine and putrescine into the cells and efficiently exchanged putrescine for ornithine. Analysis of the flanking regions showed high identity levels with different *S. epidermidis* plasmid sequences, which would confirm the plasmidic location of the *odc* operon. It follows that the *odc* and *potE* gene pair encodes a putrescine-producing pathway in *S. epidermis* 2015B that was acquired through horizontal gene transfer.

Coagulase-negative staphylococci (CNS) are often present in food-related samples and especially in fermented products (cheese or dry sausage) (4, 19, 23, 39). They are considered positive flora involved in the development of organoleptic characteristics of end products. Some strains are even used as starters (11, 41). However, although CNS are generally recognized as safe microorganisms, questions regarding presumption of safety have been raised for some CNS species which are common to human and food environments, such as *Staphylococcus epidermidis* and *S. saprophyticus* (responsible for nosocomial and urinary tract infections, respectively). One of the main risk factors identified corresponds to the ability to produce biogenic amines (BA) (33, 34, 43, 44) because of the toxicological implication of these molecules in food products (42). Histamine and tyramine, the main studied amines, can lead to more or less severe intoxications when ingested in great quantities (27) or by people with some catabolic deficiency (9). Putrescine and cadaverine are considered potentiators of the histamine and tyramine effect (21, 31). Most studies concerning biogenic amine production by staphylococci have been performed with fermented meat products (6, 33, 34, 35, 43). Other authors identified CNS strains that produced biogenic amines in other foodstuffs and especially fish-related samples (18, 22).

Biogenic amines (BA) are produced in food matrices containing free amino acids via intracellular bacterial catabolic pathways that consist of a decarboxylase and a transporter responsible for the uptake of the amino acid and the excretion of the amine. The pathways convert histidine to histamine, tyrosine to tyramine, ornithine to putrescine, etc. Alternatively, putrescine can be formed by agmatine deimination, which itself is the decarboxylation product of arginine. These two pathways may participate in the generation of metabolic energy and/or resistance against acid stress. The decarboxylation pathways are secondary metabolic-energy-generating pathways that generate proton motive force, whereas the deiminase pathways produce ATP at substrate level and ammonia (28).

The genes encoding the various decarboxylation pathways responsible for BA formation have been extensively studied in different bacterial groups and especially in lactic acid bacteria (10, 14, 15, 26, 29, 30, 32, 33). Little work has been done on the corresponding genes in CNS. Recently, histidine decarboxylase genes have been characterized in *S. capitis* (16) and *S. epidermidis* (K. Yokoi and K. Kodaira, unpublished data; GenBank accession no. AB378754), while a fragment of a putative tyrosine decarboxylase gene was described in an *S. epidermidis* strain (46). Genome sequencing has demonstrated that putrescine production by ornithine decarboxylation is a trait that is abundantly found in the Gram-negative *Gammaproteobacteria* but rarely in Gram-positive bacteria. The pathway in *Escherichia coli* is an example of a well-characterized pathway at both the genetic and physiological levels (24).

In this study, the origin of the ability of *S. epidermidis* 2015B to form putrescine and the genetic basis of strain-to-strain variation of this trait in the *S. epidermidis* species were investigated.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. epidermidis* strain 2015B was originally isolated from a mahimahi fish basket (1) and kindly provided by D. Green from the Center of Marine Sciences and Technology, Food Science Department, of the North Carolina State University. Six *S. epidermidis* strains related to dry sausage samples (VIII10B3, VII20B2, OB2, VIII 10B1, SV1, and S2) were obtained from the collection of the Association pour le Développement de l'Institut de la Viande (ADIV) (Clermont-Ferrand, France), while eight milk (S42) and cheese (S115, S118, and S119 from Camembert cheeses and S147, S148, S150, and S152 from Pont-l'évêque cheeses) isolates came from the ADRIA Normandie collection (Villers-Bocage, France). Strains were grown in tryptic soy broth yeast extract (TSBYE) in aerobic conditions with agitation or tryptone soya yeast extract agar (TSBYA) (AES, France) and incubated at 30°C. *Lactobacillus* strain 30a ATCC 33222 (obtained from the American Type Culture Collection), a histidine decarboxylase (HDC)- and ornithine decarboxylase (ODC)-positive bacterium, and *Lactobacillus brevis* IOEB 9809 (obtained from the Faculté d'Oenologie de Bordeaux, France), a tyrosine decarboxylase (TyrDC)- and agmatine deiminase (AgDI)-positive bacterium, were grown in de Man, Rogosa, and Sharpe medium (MRS) (AES, France) at 37°C and 30°C, respectively.

Preparation of template DNA. Bacterial cultures were first grown to an optical density at 600 nm (OD_{600}) of 0.6. Total DNA was then extracted from bacterial cultures using 1.5 ml of culture with the Nucleospin tissue kit (Macherey-Nagel) according to manufacturer's instructions (50 to 100 ng of DNA was used in all PCR experiments). Plasmid DNA was extracted using the Plasmid midi-kit (Qiagen) according to a user-developed protocol (http://www1.qiagen.com/literature/protocols/pdf/qp10.pdf). Plasmid restrictions were performed using the AvaI and XhoI restriction enzymes (Fermentas) according to the manufacturer's instructions.

PCR amplification. The detection of four BA-associated genes (*hdc*, *odc*, and the AgDI and TyrDC genes) was performed using a multiplex PCR method. This method corresponded to a modification of the multiplex PCR method previously described by Coton and Coton (12) targeting the TyrDC and *hdc* genes with the specific primers TD2/TD5 and HDC3/HDC4 as well as a PCR internal control (16S rRNA gene). Two new primer sets were added for detection on the one hand of *odc* genes, ODC1 (5′ NCAYAARCAACAAGYNGG 3′) and ODC2 (5′ GRTANGGNTNNGCACCTTC 3′), and, on the other hand, of AgDI genes, AgD1 (5′ CAYGTNGAYGGHSAAGG 3′) and AgD2 (5′ TGTTGNGTRATR CAGTGAAT 3′). Primer concentrations were 0.8 μM for ODC1, ODC2, AgD1, and AgD2, 0.2 μM for TD2 and TD5, 0.12 μM for HDC3 and HDC4, and 0.05 μM for BSF8 and BSR1541. All multiplex experiments were carried out in the presence of 200 μM deoxynucleoside triphosphate (dNTP) (Invitrogen), 10 μg/ml bovine serum albumin (BSA) (Amersham), and 1 U HotMaster *Taq* polymerase (5 PRIME GmbH) in a final volume of 50 μl. The amplification program was as follows: 95°C for 5 min, 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min 30 s, and a final extension at 72°C for 5 min.

Acquisition of unknown sequences adjacent to the *odc* gene was performed using a restriction site-PCR (RS-PCR) method. The method corresponded to the multiplex RS-PCR described by Weber et al. (47), with a single modification: PCR primers corresponding to the known sequence used for the second PCR were also used for sequencing. All PCR experiments were performed in a Mastercycler gradient PCR machine (Eppendorf).

Aliquots (18 μl for multiplex PCR products and 9 μl for RS-PCR-generated fragments) of each PCR sample were analyzed using 0.8% (wt/vol) agarose gels (Invitrogen, France) in 1× Tris-borate-EDTA (TBE) buffer at 130 V for 50 min and then visualized with ethidium bromide staining using a GelDoc2000 and visualized using the Quantity One software (Bio-Rad, France).

DNA sequencing and sequence analysis. The *odc* and RS-PCR products were purified using the GenElute PCR purification kit (Sigma, France) and sequenced by MWG Biotech (Germany). Alignments were performed using either the ClustalX program (45) or the Bionumerics software (Applied Maths, Belgium). Sequence comparisons against international data banks were performed using BLAST (2). Theoretical molecular weight and isoelectrical point were estimated by the "compute pI/M_w" tool (http://www.expasy.ch/tools/pi_tool.html), while conserved domains were identified by the NCBI conserved domain search tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Detection of biogenic amines by HPLC. The ability to form biogenic amines (BA) was also evaluated by high-performance liquid chromatography (HPLC). To do so, the strains were cultivated in the medium described by Bover-Cid and Holzapfel (5) except that six precursor amino acids (L-histidine monohydrochloride, L-ornithine monohydrochloride, L-lysine monochloride, L-phenylalanine, L-tryptophan [Sigma-Aldrich, France], and L-tyrosine disodium salt [BioWest, France]) were added at 2.5 g/liter each. The cultures were incubated at 30°C for 4 days with agitation. Following centrifugation (15 min, 10,000 rpm), the supernatant was filtered using 0.45-μm-pore-size filters. Biogenic amines were analyzed in filtered supernatants by reverse-phase liquid chromatography (20) of their dansyl derivates.

Cloning and expression of *potE*. The sequence encoding the putative ornithine/putrescine exchanger was amplified by PCR from total DNA with primer potE-fw (5′-GCGAAACCATGGCTGAAAAGAAAAAAATGAGTGTTACT C-3′) and primer potE-rv (5′-GCGAAATCTAGACGCTTATATATCAAGTT ATTCAAAA-3′), introducing NcoI and XbaI restriction sites. The 1.47-kb product was ligated in the NICE expression system vector pNZ8048 to yield pNZpotE-Se, which was transformed to *Lactococcus lactis* NZ9000 (17). For expression of *potE*, *L. lactis* NZ9000 cells containing pNZpotE-Se were grown in M17 medium supplemented with 0.5% glucose and induced with nisin (5 ng/ml final concentration) in mid-exponential phase. Cells were harvested after 1 h of induction.

Ornithine and putrescine transport assays. After 1 h of nisin-induced *potE* expression, cells were washed in 100 mM potassium phosphate (KPi) buffer, pH 6.0, and resuspended in the same buffer to an optical density at 600 nm (OD_{600}) of 2. Glucose was added to 0.2% (wt/vol), and 100-μl samples were incubated at 30°C with constant stirring. After 5 min of preincubation, ^{14}C-labeled ornithine (PerkinElmer) or putrescine (Amersham) was added to a final concentration of 17.5 μM or 4.5 μM, respectively. Uptake was stopped at the indicated time points by the addition of 2 ml of ice-cold 0.1 M LiCl solution, immediately followed by filtration through a 0.45-μm-pore-size nitrocellulose filter (BA 85; Schleicher & Schuell GmbH). The filter was washed once with 2 ml ice-cold 0.1 M LiCl and submerged in Emulsifier Scintillator Plus scintillation fluid (Packard BioScience), and the retained radioactivity was measured in a Tri-Carb 2000CA liquid scintillation analyzer (Packard Instrumentation). In the exchange experiment, 5 μl of 1 M unlabeled ornithine was added to the cells, 1 min after the addition of [^{14}C]putrescine.

Nucleotide sequence accession number. The nucleotide sequence determined in this work has been deposited in GenBank (accession no. GU799625).

RESULTS

Genotypic and phenotypic characterization of putrescine production by *Staphylococcus epidermidis* strains. The ability to produce biogenic amines, including histamine, tyramine, and putrescine (from either ornithine or agmatine), was evaluated in *S. epidermidis*. Total DNA isolated from 15 strains of food-related *S. epidermidis* was assayed for the presence of genes corresponding to histidine decarboxylase (*hdc*), tyrosine decarboxylase (the TyrDC gene), ornithine decarboxylase (*odc*), and agmatine deiminase (the AgDI gene) using a multiplex PCR method. This method corresponds to a modification of a previously described multiplex PCR method targeting TyrDC and *hdc* genes (12) by the addition of two new primers targeting *odc* and AgDI genes. Consensual primers ODC1/ODC2, targeting a 900-bp partial *odc* gene fragment, were designed based on the *odc* sequences of *Lactobacillus acidophilus* NCFM CP000033, *Lactobacillus gasseri* ATCC 33323 CP000413, *Lactobacillus johnsonii* NCC 533, *Lactobacillus* 30a LSU11816, and *Oenococcus oeni* AJ746165. These primers were located at positions 1116 to 1133 and 2020 to 2038 on the *O. oeni odc* gene sequence. For the agmatine deiminase pathway, a 600-bp partial AgDI gene fragment was targeted with the consensual primers AgD1/AgD2 based on known sequences of *Pediococcus pentosaceus* ATCC 25745 CP000422, *L. brevis* IOEB 9809 AF446085, *Listeria monocytogenes* 4b F2365 AE017262, and *Lactobacillus sakei* 23K CR936503. These primers were located at positions 478 to 494 and 1060 to 1078 on the *P. pentosaceus* AgDI gene sequence. Only in the *S. epidermidis* 2015B strain, amplification of a specific band with a size comparable to the one of *Lactobacillus* 30a (~900 bp), used as a positive control (Fig. 1), was observed.

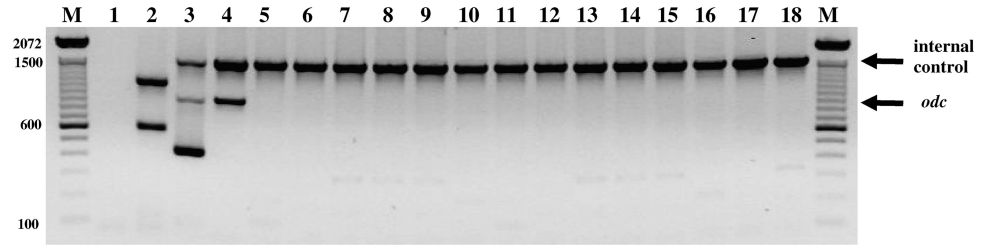

FIG. 1. Multiplex PCR-based detection of biogenic amine-associated genes. M, 100-bp ladder. Lanes: 1, H_2O negative control; 2, positive control *L. brevis* IOEB 9809 (positive for the TyrDC and AgDI genes); 3, *Lactobacillus* 30a (odc^+ hdc^+); 4 to 18, *S. epidermidis* strains 2015B, VIII10B3, VII20B2, OB2, VIII 10B1, SV1, S2, S152, S118, S115, S148, S119, S150, S42, S147.

The ability of the 15 strains to form biogenic amines *in vitro* was evaluated on the improved detection medium (5) supplemented with six precursor amino acids (histidine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine) and measured by HPLC. The *S. epidermidis* 2015B culture supernatant was the only one to contain significant amounts of biogenic amines, namely, putrescine (1,460.85 μg/ml) and, to a lesser extent, cadaverine (146.92 μg/ml). Remarkably, while *S. epidermidis* 2015B (isolated from a mahimahi fish basket) was described as a histamine-forming strain (1), only a very minor amount of this amine (4.03 μg/ml) was produced. Two strains, namely, OB2 and S42, produced minor amounts of tryptamine (6.01 and 14.00 μg/ml, respectively) and phenylethylamine (26.10 and 26.34 μg/ml, respectively).

To localize the putrescine production pathway, plasmid DNA was extracted from the *S. epidermidis* 2015B strain followed by digestion using the restriction enzyme AvaI or XhoI. After electrophoresis, a PCR using the *odc* primer set was performed on each of the obtained bands by directly targeting each band in the gel as DNA matrix. For both the AvaI and XhoI restrictions, the top band allowed for the amplification of the *odc* fragment (Fig. 2). The results strongly suggest that a catabolic ornithine decarboxylation pathway encoded on a plasmid is responsible for putrescine formation by *S. epidermidis* 2015B.

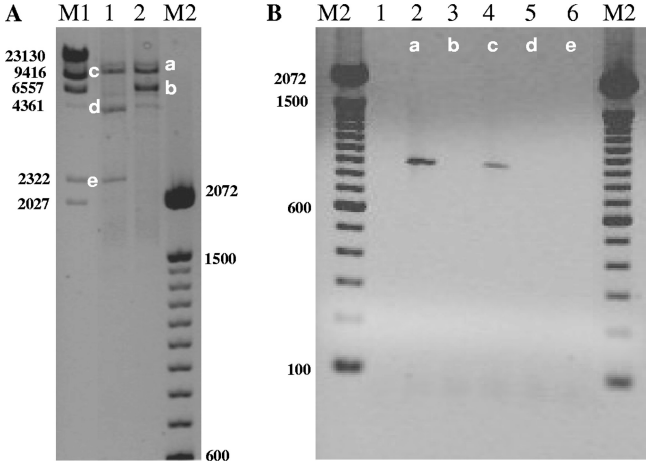

FIG. 2. (A) Results of enzymatic restriction performed on *S. epidermidis* 2015B plasmid extraction. Lanes: M1, DNA marker phage λ/HindIII; 1, digestion by AvaI; 2, digestion by XhoI; M2, 100-bp ladder. (B) PCR-based detection of *odc* gene. Lanes: M2, 100-bp ladder; 1, H_2O negative control; 2 to 6, enzymatic restriction bands a to e, respectively, used directly as DNA matrix.

Characterization of the *odc* region. The obtained PCR fragment identifying the *odc* gene in *S. epidermidis* 2015B was sequenced and consisted of 832 bp. In order to determine the complete sequence of the *S. epidermidis* 2015B *odc* gene as well as of its flanking regions, a multiplex restriction site-PCR (RS-PCR) method, based on the one proposed by Weber et al. (47), was used. The method allows for rapid acquisition of unknown DNA sequences adjacent to a known segment in both the 5′ and 3′ directions. The repetitive use of this method by the creation of new sets of primers based on each newly determined sequence allowed us to obtain a 6,292-bp-long nucleotide sequence (GenBank accession no. GU799625) from the original 832-bp *odc* partial sequence. Sequence analysis of the fragment revealed the presence of three complete open reading frames (ORFs) flanked by two partial ORFs (Table 1).

The *odc* gene consisted of 2,175 bp encoding a 724-amino-acid protein exhibiting an ornithine decarboxylase family conserved domain. Sequence identities on the order of 60 to 65% were observed with the known ornithine decarboxylases of Gram-positive bacteria, i.e., *Oenococcus oeni* (AJ746165) and *Lactobacillus* 30a (LSU11816), as well as ODCs of Gram-negative bacteria, including *Haemophilus influenzae* (L42023) and *Dichelobacter nodosus* (CP000513). Comparison of the translated sequence to the ODC sequence of *Lactobacillus* 30a, for which the crystallographic structure has been determined (37), indicated that the residues involved in enzymatic activity were conserved (31, 38), including the pyridoxal-5-phosphate binding domain and the residue involved in the association of dimers into dodecamers. Although the *S. epidermidis* 2015B ODC showed the highest percent identity with the *O. oeni* ODC (GenBank accession no. CAG34069), it lacks the N-terminal extension of the latter (31).

Immediately downstream of the *odc* gene at an intergenic distance of only 22 bp, and therefore certainly cotranscribed, an open reading frame encoding a 442-amino-acid-long protein was found. The putative protein shares 59% sequence identity with the functionally characterized putrescine-ornithine antiporter (PotE) of *E. coli.* Homologues of PotE are found downstream of the ODC-encoding genes of *O. oeni* (CAM07323) and various Gram-negative bacteria as well (Fig. 3). It is likely that the two genes form a single transcriptional unit encoding the ornithine decarboxylation pathway. The same operon structure is not found around the *odc* genes found in a group of lactobacilli. Rather, an amino acid trans-

porter distantly related to PotE's is located upstream of the decarboxylases. Moreover, the decarboxylases and transporters are divergently transcribed. The *S. epidermidis* PotE shares highest sequence identities with the transporters of *O. oeni* and *H. influenzae* (69 and 67%, respectively).

Downstream of the putative *potE* gene is an ORF (*cep*) encoding a 306-amino-acid protein that is a member of the cation efflux protein superfamily. Members of this family are integral membrane proteins that are found to increase tolerance to divalent metal ions such as cadmium, zinc, and cobalt by excretion of these ions from the cell. The translated sequence exhibited 70% and 66% identities with proteins identified in *S. epidermidis* (BAG55998) and *S. capitis* (CAK55534), respectively. Interestingly, in both cases, the proteins were found associated with another biogenic amine production pathway, the histidine decarboxylation pathway (GenBank accession no. AB378754 [Yokoi and Kodaira, unpublished], GenBank accession no. AM283479 [16]). The role of this protein in the context of biogenic amine production needs further investigation.

The three genes on the sequenced fragment are flanked upstream by a partial sequence encoding 93 amino acids exhibiting high identity levels (95% to 100%) with a mobilization protein (MobC) identified on various *S. epidermidis*-associated plasmids (GenBank accession no. ZP_04826590, ZP_04796278, NP_976276, and NP_863195). Downstream they are flanked by a partial sequence encoding 60 amino acids that are 100% identical with an IS*431mec*-like (methicillin resistance-associated insertion sequence-like) element transposase (*tra*) identified in various *Staphylococcus* species, including *S. epidermidis* (i.e., GenBank accession no. YP_187845, YP_187537, and NP_763645). The high level of identity observed for the translated sequence with transposases associated with the transfer of methicillin resistance in various *Staphylococcus* species suggests a specificity of this protein for the *Staphylococcus* genus.

Functional expression of *potE* in *Lactococcus lactis*. The *potE* gene of *S. epidermidis* was cloned in the NICE expression system vector pNZ8048 (plasmid named pNZpotE-Se) for nisin-inducible expression in *L. lactis* NZ9000 (17). At a concentration of 17.5 μM ^{14}C-ornithine, the host cells containing the empty vector pNZ8048 took up ornithine at an initial rate of 2.7 nmol · min^{-1} · mg^{-1}, demonstrating the presence of an endogenous ornithine transport system in *L. lactis* NZ9000 (Fig. 4A). *L. lactis* cells expressing the *S. epidermidis* transporter gene showed a higher initial rate of ornithine uptake of 5.0 nmol · min^{-1} · mg^{-1}, demonstrating that *potE* encoded an ornithine transport protein. Even more convincing was the uptake of putrescine at an initial rate of 7.8 nmol/min · mg at a concentration of 4.5 μM by the recombinant strain, while the host strain did not take up significant amounts of [^{14}C]putrescine (Fig. 4B, circles).

The function of PotE in the ornithine decarboxylation pathway is the combined uptake of ornithine and excretion of putrescine. To demonstrate that the transporter catalyzes ornithine/putrescine exchange, cells were allowed to take up ^{14}C-labeled putrescine until a plateau was reached, after which an approximately 200-fold excess of unlabeled ornithine was added. The result was a rapid release of [^{14}C]putrescine from

TABLE 1. *odc* region putative encoded proteins

Gene	Location in nucleotide sequence	G+C (%)	Predicted protein (aa/kDa/pI)[a]	Conserved domain[b]	Closest protein (aa)	Proposed function	% identity[c]	Accession no.	Organism
mobC	0–282	43.6		None	Mobilization protein MobC	Mobilization protein	100 (first 72 aa)	ZP_04826590	*Staphylococcus epidermidis* BCM-HMP0060
odc	910–3084	29.0	724/83.8/5.3	Ornithine decarboxylase family	Ornithine decarboxylase	Ornithine decarboxylase	65	CAG34069	*Oenococcus oeni* RM83
potE	3107–4435	31.7	442/47.3/9.4	Amino acid transporter	PotE protein	Ornithine/putrescine antiporter	69	CAM07323	*Oenococcus oeni* RM83
cep	4599–5519	24.2	306/35.0/6.6	Cation efflux family	Hypothetical protein	Unknown	70	BAG55998	*Staphylococcus epidermidis* TYH1
tra	6108–6292	35.1		Integrase core domain	IS*431mec*-like transposase	Transposase	100	YP_187845	*Staphylococcus epidermidis* RP62A

[a] Amino acids (aa), theoretical molecular mass, and isoelectrical point, the last two as estimated by the "compute pI/M_w tool" (http://www.expasy.ch/tools/pi_tool.html).
[b] Identified by the NCBI conserved domain search tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).
[c] Identical amino acid percentage between the predicted sequence and the closest sequence in GenBank using BlastP.

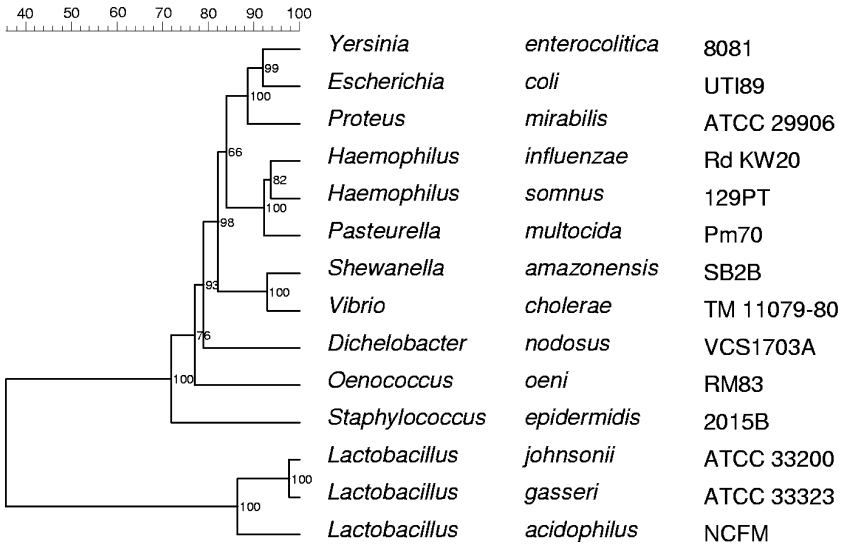

FIG. 3. Phylogenetic tree comparing the protein sequences of amino acid transporters associated with ornithine decarboxylases. The tree was constructed using the Pearson correlation; unweighted-pair group method using average linkages (UPGMA) and bootstrap values are indicated at branch points (percentages of 1,000 replications).

the cells, showing the exchange mode of transport (Fig. 4B, triangles).

DISCUSSION

Concerning biogenic amine production by staphylococci, several studies have been performed in various foodstuffs. In fermented meat products, the results obtained are contrasted; while the ability of strains of *S. carnosus* or *S. xylosus* to form BA was reported in dry sausages (34, 35, 43), other studies indicated that no or few BA producers were found among CNS strains in the same type of products (6, 33). In fish-related samples, BA-producing CNS strains were also observed and belonged mainly to the *S. epidermidis* and *S. capitis* species in salted anchovies (22) and *S. hominis* in temperature-abused tuna (18). BA production by CNS in dairy products does not seem to constitute a safety problem so far (25), although Straub et al. (44) reported that an *S. epidermidis* strain isolated from a cheese surface was able to produce BA.

In this study, the genotypic and phenotypic characterization of food-related *S. epidermidis* isolates for biogenic amine production indicated that only 1 out of 15 strains was able to produce significant amounts of biogenic amines (putrescine and, to a lesser extent, cadaverine), thus indicating the existence of a strain-to-strain variation in the ability to form BA in this species.

Some authors have already shown the existence of a strain-dependent ability to form BA in various bacterial species. For instance, the ability to form histamine was shown to be strain dependent in *O. oeni* (14) and *Lactobacillus hilgardii* (30). In the latter case, the amine-producing pathway was harbored by an unstable plasmid (30), whereas for tyramine production, the presence of a putative genomic island harboring the complete TyrDC operon was shown to be associated with the ability to form tyramine in *L. brevis* (13). Concerning putrescine production, Marcobal et al. (32) recently demonstrated that the ability to form putrescine in *O. oeni* was a strain-dependent trait and that the involved genes, the ornithine decarboxylase, *odc*, and the putrescine-ornithine antiporter, *potE*, were situated at the chromosomal level in a region that was certainly acquired through horizontal gene transfer (HGT).

For *S. epidermidis* 2015B, the presence of a sequence corresponding to a mobilization protein (MobC) was observed upstream of the *odc* gene. In *S. aureus*, MobC has been shown to correspond to a nicking accessory protein belonging to the relaxosome complex, also constituted by the MobA (DNA relaxase) and MobB (mobilization accessory protein) proteins, that allows for the preparation of plasmid transfer between bacterial cells by conjugation. MobC proteins have been shown to possess binding specificity for the origin of transfer (*oriT*) and therefore contribute to the site- and strand-specific cleavage at a unique nick site (*nic*) within the *oriT* performed by the relaxosome (8). The obtained *mobC* sequence, also found on various *S. epidermidis* plasmids, suggested that the putative putrescine production pathway could be carried by a plasmid. Comparison to an international data bank of the 628-bp intergenic sequence situated between the putative *mobC* and *odc* genes indicated that the 380 leftmost base pairs presented high identity levels (from 91 to 96%) with regions of *S. epidermidis* plasmids (i.e., GenBank accession no. SEU40259 and GQ900505). For the intergenic sequence situated between the putative cation efflux protein and IS*431mec*-like transposase gene, the 306 leftmost base pairs showed a high degree of identity (>91%) with *S. epidermidis* plasmidic fragments (i.e., GenBank accession no. GQ900505 and AF045240). Therefore, the obtained sequences as well as experiments performed on the plasmid extraction confirmed that the putrescine production pathway, consisting at least of the *odc* and *potE* genes, is carried by a plasmid in *S. epidermidis* strain 2015B and was thus acquired through HGT. This localization explains the fact that the putrescine production trait is observed to be strain dependent in the *S. epidermidis* species.

Remarkably, as already observed for *O. oeni* (32), the ge-

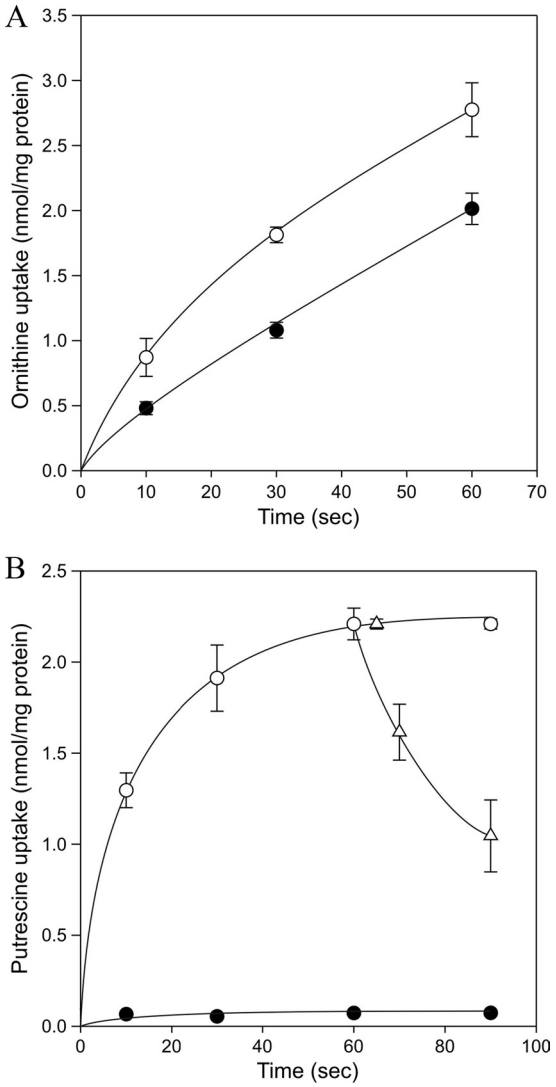

FIG. 4. Ornithine (A) and putrescine (B) uptake in *Lactococcus lactis* NZ9000 harboring pNZpotE-Se (open circles) or pNZ8048 (closed circles). Cells were induced with 5 ng/ml nisin for 1 h for *potE* expression. ^{14}C-Ornithine and [^{14}C]putrescine were added to final concentrations of 17.5 μM and 4.5 μM, respectively. Panel B also shows the release of ^{14}C-putrescine from *potE*-expressing cells by external addition of 1 mM unlabeled ornithine, 1 min after ^{14}C-putrescine uptake (open triangles). The data are the means ± standard deviations of results of two independent experiments.

netic organization as well as sequence identity levels of the *odc* and *potE* genes indicated that the putrescine-forming pathway in *S. epidermidis* 2015B is more related to those described in Gram-negative bacteria than to those in phylogenetically closer bacteria such as Gram-positive bacteria and especially lactic acid bacteria.

It is also interesting to note that the PotE-encoding gene of *S. epidermidis* 2015B is only the second one identified in a Gram-positive bacterium. The transport properties of *S. epidermis* PotE expressed in *L. lactis* support the involvement of the *odc-potE* pathway in putrescine formation by *S. epidermidis* 2015B. The transporter catalyzes exchange of putrescine and ornithine.

The role of biogenic formation by amino acid decarboxylation is believed to protect against acid stress and/or to generate metabolic energy (48). The pathways that consist of a decarboxylase and an amino acid/biogenic amine exchanger generate proton motive force through proton consumption in the cytoplasmic decarboxylation reaction and membrane potential generation through electrogenic exchange of the precursor amino acid and the corresponding biogenic amine (36). In this context, Azcarate-Peril et al. (3) identified the *odc* gene to be involved in acid tolerance in *L. acidophilus* NCFM by challenging mutants with a variety of acidic conditions, and Pereira et al. (40) demonstrated the involvement of ODC in proton motive force generation. Bover-Cid et al. (7) indicated that ornithine decarboxylase in *Lactobacillus curvatus* CTC273 would not seem to be a mechanism to neutralize the acid environment but may rather play a role in supplying metabolic energy.

Finally, the presence, downstream of the putrescine production pathway, of a cation efflux protein-encoding gene involved in detoxification action towards toxic metallic ions raises the question of the existence of a resistance island (combining the putrescine production pathway for acid stress resistance and the cation efflux protein for tolerance to toxic metallic ions), harbored by a plasmid, in *S. epidermidis* 2015B. The effect of the presence of these genes on the adaptability and growth of *S. epidermidis* 2015B in various environmental conditions should be further investigated.

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